Synovial Fluid Crystals and Cartilage Proteoglycans of Arab Patients with Osteoarthritis of the Knee Joint

M. K. M. Abdelnabi, A. M. Ashmawee, F. S. Mohammed, G. M. Kamaludin

The pattern of osteoarthritis in Arabs is clinically and radiologically different to that in Caucasians and Africans. Crystal deposition has been proposed as a possible factor in the production or exacerbation of osteoarthritis. This study included 50 Arab patients (25 males and 25 females) with primary osteoarthritis of the knee joint with effusion. The patients had no other cause for the effusions. Five types of crystals were identified in 44% of patients by scanning electron microscopy and polarized light microscopy. The crystals were calcium hydroxyapatite 50%, calcium pyrophosphate 27%, dicalcium phosphate dihydrate 4.5%, calcium oxalate 4.5% and monosodium urate monohydrate 4.5%. A mixture of crystals was found in 9% of patients. Cartilage proteoglycans were shown to be less aggregated in patients with crystal deposition. The serum pH was normal for all patients. Synovial effusion pH was slightly higher (7.40-7.48) in patients with crystals. The prevalence of hydroxyapatite crystals in the synovial fluid and high female to male ratio (1.75:1) are strongly correlated with the advancement of clinical picture and radiological findings. These factors in addition to obesity and different habits of daily life may explain the relatively high incidence of osteoarthritis of the knee joint among Arab patients.

Adequate information exists on the pattern of osteoarthritis (OA) of the knee in Western1-2 and African countries.3 There are few reports from Saudi Arabia and the Gulf region.4

Primary OA of knee joint, but not the hip, is a common medical problem among Arab patients. A Swedish Saudi study4 reported different patterns of OA. In Saudi Arabs 40% are under age of 50 years, while in Sweden OA is rare under that age. The Arab female to male ratio was 2.3:1 (Caucasian 4:1). The OA was bilateral in 92% of Arab patients. Involvement of patellofemoral articulation occurred
in 80% of Arabs and in only 48% of Swedes. Varus deformity is seen in 83% of Arabs but not in a single Arab patient had OA of the hip joint. On the other hand the OA patients of any kind exhibited a high incidence of hip replacement in a Finnish study where OA patients were 80% obese. Hip OA in Hong Kong is 0.6% for males, 0.0% for females and 0.1% for an Indian population. The prevalence of crystal deposition to the pathogenesis of OA remains unclear. However, it induces exacerbation of OA. Differences in decade of onset, ratio of male to female incidence and severity of arthritis suggest different in genetic defects. Therefore, comparative biophysical studies are helpful in testing the various hypotheses concerning aetiology and pathogenesis of OA.

This study presents data from scanning electron microscopy and polarized light microscopy on types and prevalence of crystals among Arab patients suffering from OA of the knee.

Patients and Methods
We investigated 50 patients (25 males and 25 females) with an average age of 55 years, both males and females, suffering from primary osteoarthritis of the knee joints with non-acute effusions. The patients were selected consecutively at random from the out-patients clinics. The diagnosis was based on clinical and radiological features as described. Radiograms were taken in anteroposterior views with the patients non-weight bearing and weight bearing. Lateral views were taken with the knee in 45° flexion. Axial views of the patellofemoral joint were taken with the knee flexed 20°. Radiograms were read and graded on a 1–4 scale. X-rays of both hips were taken. All patients had a normal CBC, ESR, serum creatinine, uric acid, calcium, magnesium, alkaline phosphatase, free thyroxine, and negative RA factor and CRP.

Scanning electron microscopy
We used a Jeol combined Electron Microscope Tmscan. We centrifuged the synovial fluid at 300 rpm. The precipitate was air dried on a carrier of copper column. The sample on the carrier was coated by a gold sputter coating. The carrier was investigated on the stage of an electron microscope. For the morphology of crystals, we used a low magnification image with large focal depth. For proteoglycans aggregation we used x 80 000 magnification.

Synovial fluid
Synovial fluid was collected as described. The absolute leukocyte count of the synovial fluid effusion was calculated (absolute white blood cell count = leukocyte/mm³ × aspirated volume in mm³). Patients with a leukocyte count more than 2000/mm³ were excluded because of the possibility of coexisting diseases. A wet preparation slide was made for microscopic study. The pH was measured by a digital pH/MV and temperature meter model 7065.

Polarized light microscope
To an aliquot of synovial fluid, 0.03 cc of hyaluronidase was added, followed by centrifugation at 1000 rpm for 10 min. The sediment was examined for birefringent crystals.

Proteoglycans
Fragments of articular cartilage were found in the synovial fluid and prepared for the proteoglycans microscopic study.

Statistical analysis
We used paired t-tests and correlation coefficients; p < 0.05 was considered significant.

Results
Crystals in synovial effusion
We identified five types of crystals in 22 patients (44%). The female to male ratio was 1.75:1. The age range for males was 48–65 years and 45–60 years for females. The difference was statistically significant, p < 0.001 (Table 1) and (Figs 1 and 2).

Hydroxyapatite crystals (HA)
These were found in 50% of patients with crystal deposition. The crystals were rod or needle shaped either uniformly dense or with fewer dense centres. Some crystals were found densely packed in 'coin-like' clumps. Others were loosely scattered in an amorphous electron-dense material.

Calcium pyrophosphate dihydrate crystal (CPPD)
These were found in linear and rhomboidal forms in 27% of patients with crystals. The linear form is rod-like with blunt ends, in contrast to the more sharply pointed ends of urates. CPPD crystals were weakly or non-birefringent. Dicalcium pyrophosphate dihydrate crystals were found in one patient.
Table 1

Presence and type of crystals found in synovial effusion of osteoarthritic knee joint of Arab patients

<table>
<thead>
<tr>
<th>Patients No.</th>
<th>HA</th>
<th>CPPD</th>
<th>DCPPD</th>
<th>Ca.Ox.</th>
<th>MSU</th>
<th>HA + CPPD</th>
<th>HA + CPPD + MSU</th>
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<tr>
<td>Males</td>
<td>8</td>
<td>16</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Females</td>
<td>14</td>
<td>28</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>44</td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Hydroxyapatite (HA), calcium pyrophosphate dihydrate (CPPD), dicalcium pyrophosphate dihydrate (DCPPD), calcium oxalate (Ca.Ox), monosodium urate (MSU).*

Figure 1. Electron microscopy pictures of crystals. (a) Hydroxyapatite rod or needle shaped and densely packed in 'frond-like' clump. (b) HA, scattered in an amorphous electron dense material × 40 000. (c) Calcium pyrophosphate dihydrate crystals, the linear form, rod-like with blunt ends × 20 000. (d) Dicalcium phosphate dihydrate crystals × 20 000. (e) Collagen fibres and HA crystals in the synovial fluid × 30 000. (f) Decreased aggregates of cartilage proteoglycans × 80 000.
Monosodium urate crystals (MSU)
Needle shaped crystals of 3 μ to 20 μ show strong birefringence.

Calcium oxalate
This was seen as pleomorphic crystals with large bipyramidal forms showing strong birefringence in a positive direction. A mixture of HA, CPPD and MSU crystals was found in one patient. An association of HA and CPPD was also found in another patient.

Table 2
Correlation between radiographic grade of OA, and presence of HA or CPPD crystals in the knee joint

<table>
<thead>
<tr>
<th>O/A grade</th>
<th>Patients no.</th>
<th>No. (%) with crystals*</th>
<th>Average age of patients with crystals</th>
</tr>
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<tr>
<td>1</td>
<td>12</td>
<td>3 (25%)</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>7 (39%)</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>8 (53%)</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>4 (80%)</td>
<td>60</td>
</tr>
<tr>
<td>TOTAL</td>
<td>50</td>
<td>22 (44%)</td>
<td>53</td>
</tr>
</tbody>
</table>

*Significant positive correlation, between higher grade of OA and presence of crystals (p<0.001).

Proteoglycans
The proteoglycans were found to be less aggregated in OA patients with crystal deposition.

pH value
Serum pH was normal. In synovial fluid with crystals, pH was higher (7.40–7.48), but normal in patients with MSU.

X-ray findings
Patients with crystal deposition had radiographic evidence of more severe OA changes; 40% of patients above the age of 50 developed grade 3 and 4 OA changes. None of the patients showed osteoarthritic changes in the hip joints (Table 2).

Discussion
We identified five types of crystals. The electron probe analysis calculates rate of calcium and phosphorus in the particles, which helps in differentiating apatite from pyrophosphate. Apatite-containing deposits usually appear as globular or irregular shadows of calcific density with no trabecular pattern, and lacking the linear appearance of pyrophosphate. This study found a high incidence (50%) of HA crystals in joints of Arab patients
without elevated synovial fluid leucocyte counts. The female to male ratio was 2.6:1.

Apatic and calcium phosphates were identified in synovial fluid, synovial membrane and cartilage of patients with OA. McCarty in the USA, found HA in 29% of a variety of non-inflammatory joint fluids. Dieppe in England identified CPPD and HA crystals in 41% of OA synovial fluids. Schumacher in the USA, found crystals in 60% of OA patients. This included HA in 30%, CPPD in 22% and both in 80%.

Injection of hydroxyapatite crystals into a dog's knee produces inflammation of the joint. In man, HA crystals were seen in acute undiagnosed arthritis and in exacerbation of OA. They can induce synovitis similar to that seen with urate and pyrophosphate crystals in gout and pseudogout.

Therefore, the increased frequency of HA crystals deposition in the knee joint, high female to male ratio and the strong positive correlation of these findings with radiological changes, are features which accompany the high incidence of OA of knee among Arabs.

We found CPPD crystals in 27% of Arab patients with crystals. The female to male ratio was 2:1. The incidence was a little higher than that of an American study. A hereditary chondrocalcinosis has been described in families from Canada, Czechoslovakia, Holland, Spain, Chile, France, and Sweden. Familial predisposition to crystal formation has been reported independently for MSU in gout, CPPD in pyrophosphate arthropathy and apatite in idiopathic chondrocalcinosis and calcific periarthritis. The mechanism of crystal deposition in these diseases is poorly understood. The genetic factors that enhance susceptibility have not been determined. But it seems that some heritable connective tissue factors, may be important in permitting development of the disease. Calcification of cartilage may contribute to cartilage damage by mechanical forces. These crystalline deposits induce a chronic synovitis that may increase cartilage degradation by the production of lysosomal proteases.

MSU crystals were found in one male patient. Our patient had no evidence of hyperuricaemia nor hyperuricosuria. Others found MSU during asymptomatic periods of gout, and even in joints that have never been overtly affected by gout. A mixture of HA, CPPD and MSU was found in a female aged 61 years. An association of HA and CPPD was found in a male aged 58 years. Similar associations were found in other races.

Calcium oxalate crystals deposition is a cause of calcific synovium in patients on dialysis. We detected them in one woman who was not on dialysis. Others found calcium oxalate in acute and chronic effusion, particularly in patients on diets supplemented by vitamin C.

Proteoglycan aggregates were apparently decreased among Arab patients with positive crystal deposition. In OA, synovial catabolic proteins inhibit proteoglycan synthesis. Also, enzymes capable of degrading proteoglycans and collagen are increased. Then the released proteoglycans act as a major stimulus to the chronic and immunological synovitis seen in OA. Almost two-thirds of the normal proteoglycans are present as aggregates. These proteoglycan aggregates inhibit precipitation of calcium salt and MSU. Intact proteoglycans protect cartilage from calcification with hydroxyapatite.

Electron microscopy identified three samples of synovial effusion containing collagen fibres of cartilage. These collagen fibres may act as crystal inducers, as they do in bone, and promote crystal deposition in the joints.

Rises of pH through a range of 7.2-7.4, will rapidly increase the formation of crystals in vitro. The pH of the synovial fluid in our patients with crystal deposition was high. This may be one dynamic factor responsible for the formation of crystals among our patients.

This study provides data on the joint crystals in Arab patients with knee OA. It shows the prevalence of hydroxyapatite crystals. Females were more affected and at a younger age than were males. The proteoglycan aggregates were much less. The degenerative radiographic changes correlated strongly with the presence of crystals in knees. These findings, with the effects of obesity, mechanical and environmental factors, may contribute to the high incidence of osteoarthritis of the knee joint among Arab patients.

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References
Osteoarthritis of the Knee Joint


